Basic Molecular and Cell Biology

The cell nucleus

R A LASKEY

The cell nucleus is the information centre of the cell. It is responsible for copying highly selected regions of the genome into ribonucleic acid (RNA) and for supplying precisely regulated amounts of specific RNA molecules to the cytoplasm, where they are translated into proteins. In addition it must duplicate its entire structure every time the cell divides.

This article considers how the nucleus is organised to perform the immensely complex task of selective information retrieval. Recently several different experimental approaches have elucidated how deoxyribonucleic acid (DNA) is organised in the nucleus and how specific regions of DNA are selected for expression at particular times and in particular cells of the body. One of the key problems in selective information retrieval in the nucleus becomes obvious if we consider how densely DNA is packed into the nucleus. Each chromosome consists of an individual double helix of DNA about 40 mm long by 2 nm wide packed into a nucleus of about 6 μm in diameter. The implications of these dimensions can be seen more clearly by a simple scale model enlarged one million times. On this scale the DNA in each chromosome would resemble thin string, 2 mm in diameter but 40 km long, and the total DNA in one nucleus would reach from London to Leningrad. Yet on the same scale this 1800 km of DNA must all be packaged into a nucleus of only 6 m diameter in such a way that specific regions must remain fully accessible for highly regulated expression.

The answer to how such a densely packed structure can function in selective information retrieval lies in a precise three dimensional nuclear architecture. The contents of the nucleus are not just a randomly mobile solution but are highly organised into a hierarchy of ordered structures.

Chromatin and chromosomes

The fundamental unit of DNA packaging in higher organisms is the nucleosome, in which 146 base pairs of DNA are wrapped twice around an octamer of histones, consisting of two each of histones H2A, H2B, H3, and H4. These small basic proteins neutralise the acidic charges of DNA and they also shorten it by providing spools on which the DNA is coiled. Recently it has been possible to crystallise nucleosomes and hence to determine their three dimensional structure.

A fifth histone, called H1, occupies the site at which DNA enters and leaves the nucleosome. When H1 is present nucleosomes can coil into a solenoid with six nucleosomes in each turn. This has two important consequences. Firstly, coiling compacts DNA so that it can be divided between progeny cells at mitosis, and, secondly, it allows selected regions of DNA to be folded away so that only a specific subset of the genetic information is available for expression

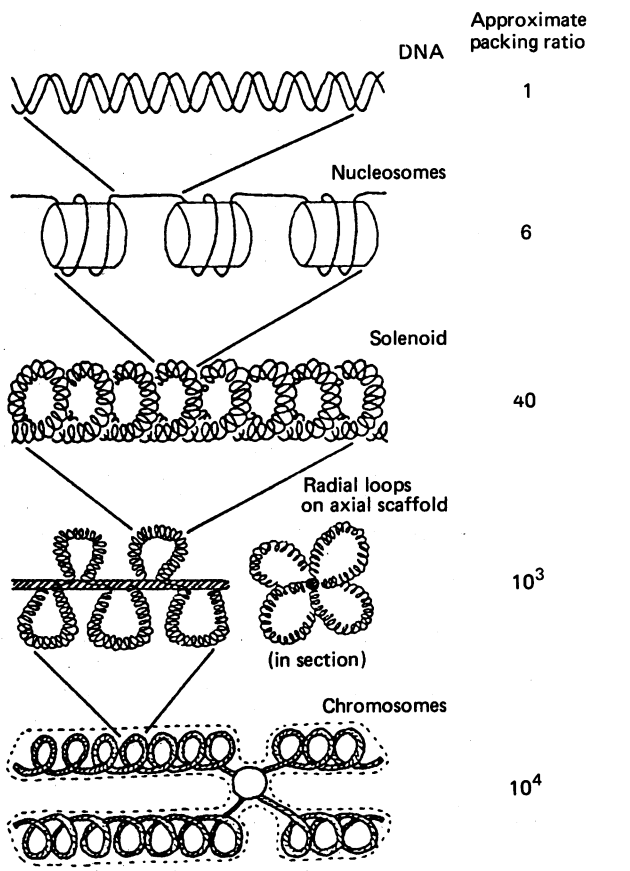

FIG 1—Summary of structural models for the packing of DNA into metaphase chromosomes. (Reprinted with permission from Laskey, 1986.)

in a particular type of cell. Active genes are packaged into a different, and more accessible, chromatin conformation from that of inactive genes in the same cell. Thus nucleosomes allow a structural level of gene regulation, by allowing selective access to regions of DNA. In addition, the absence of a nucleosome or a small group of nucleosomes from the region of DNA just upstream of a gene can be important in gene regulation. These regions are known as DNase 1 hypersensitive sites because of their extreme susceptibility to nuclease cleavage. They frequently correspond to regions of DNA which are essential for correct gene regulation (see below).

Not only is the DNA in each chromosome packaged into nucleosomes but it is also organised into a series of loops which extend radially from an axial scaffold. Loops vary in size but on average they contain 20 000-100 000 base pairs each. Specific proteins bind to and grip DNA sequences which define the bases of these loops. This level of organisation persists when chromosomes are condensed for cell division or when they are decondensed for transcription and replication during interphase. Furthermore, the

Cancer Research Campaign Molecular Embryology Research Group, Department of Zoology, Cambridge CB2 3EJ
R A LASKEY, FRS, professor

organisation does not appear to change when cells differentiate. Instead it appears to define a basal level of organisation within the nucleus (fig 1).

Each chromosome consists of a linear chain of genes, but it also contains other structural features, including centromeres and telomeres. Centromeres attach chromosomes to the spindle during mitosis and meiosis, whereas telomeres form the termini of chromosomes. Both centromeres and telomeres have been isolated and characterised by selecting for these functions on artificial chromosomes in yeast. Centromeres allow plasmids to segregate stably to progeny cells during mitosis and meiosis, whereas telomeres allow linear DNA molecules to complete their replication correctly. Without a specialised telomere DNA polymerases would be unable to replicate right to the ends of the linear chromosome, so it would become slightly shorter each cell generation. The telomeric DNA sequence is a simple repeat which can fold back to form a hairpin loop. A specialised enzyme can add DNA of this simple repeating sequence to the end, restoring or even extending the length of the chromosome without having to copy the template sequence.

The ability to construct artificial chromosomes in yeast has allowed components essential for chromosome function to be identified. Apart from centromeres, telomeres, and genes, yeast chromosomes also require specific DNA sequences to allow them to replicate. Recent evidence has shown that these "autonomously replicating sequences" are sites at which DNA replication starts in yeast. Obviously we would like to know more about equivalent sequences from mammalian cells, and an active search is in progress.

Gene expression and its control

The opportunity to clone genes and to reintroduce them into intact cells has allowed rapid advances in our understanding of gene expression and its regulation. It is possible to delete regions of DNA from within or around genes and to reclone the truncated genes in order to assay their expression. In this way the DNA sequences responsible for regulated expression of a wide range of genes have been identified.

In many cases regulatory sequences have been mapped to the flanking DNA which lies just upstream of the transcription start, though there are important exceptions in which regulatory information lies downstream or even within the transcribed region of a gene. Some upstream flanking sequences are found to be conserved between many genes whereas others confer tissue specificity. For example, the upstream regulatory region of the metallothionein gene contains a widely conserved TATA box, about 30 nucleotides before the transcription start, but it also contains three specific regulatory sequences, two of which mediate induction by heavy metals and one of which confers regulation by glucocorticoids. This third site binds the hormone receptor and thus confers hormonal control.

Several other proteins have now been discovered which act as specific transcription factors. They bind to regulatory sequences adjacent to specific genes and enable those genes to be transcribed. In general they occur at about 10 000 copies per cell, which poses an immediate problem. If each gene needed 10 000 copies of its own specific transcription factor the concentration of factors within the nucleus would be enormous. This seems to be partly solved through the use of different combinations of shared factors for some genes. This may be particularly common in "housekeeping" genes, which are required to be active in all types of cell.

The first animal cell transcription factor to be isolated and characterised showed a surprising structural feature. Its amino acid sequence contains a repeated motif of amino acids which could bind zinc. There is growing evidence that the protein is folded into a series of nine fingers which can bind to DNA and each of which contains an atom of zinc. Since this discovery similar motifs of metal binding amino acids have been observed in many other proteins which bind to DNA, suggesting that the "zinc finger" may be an important means of recognising nucleic acid sequence information.

The role of the nuclear envelope in traffic control

The nucleus is surrounded by two complete layers of membrane, but these do not exclude small molecules because the membranes are perforated at frequent intervals by nuclear pore complexes. These are grommet like structures which allow the free passage of small molecules up to the size of small proteins, but they appear to regulate selectively the transit of larger molecules in both directions. Thus above about 50 kilodaltons molecular weight only "nuclear" proteins can enter the nucleus, and other proteins are excluded.

The information which specifies selective entry resides in a very short stretch of amino acids. In the case of SV40 T antigen this sequence is: proline, lysine, lysine, lysine, arginine, lysine, valine. When a short synthetic peptide of this sequence is cross linked to cytoplasmic proteins it causes them to accumulate in the nucleus. The nuclear pore has been identified as the route of protein entry by coating colloidal gold particles with a nuclear protein and using electron microscopy to follow their fate after injection into cytoplasm. The gold particles can be seen aligned through the centre of the nuclear pore in figure 2.

The nuclear pore is also implicated in export of ribonucleo-

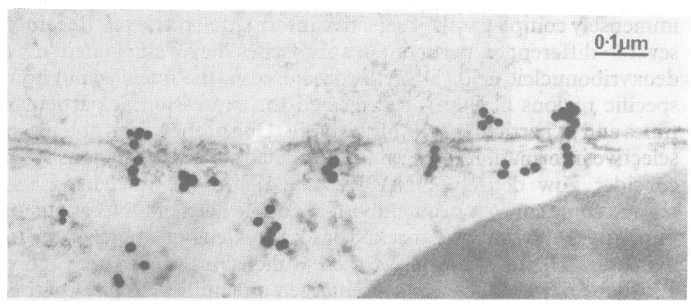

FIG 2—Electron micrograph of a section through the nuclear envelope of a frog oocyte which has been injected with colloidal gold particles coated with the nuclear protein "nucleoplasmin." Gold particles can be seen aligned through nuclear pore complexes. (Courtesy of A D Mills.)

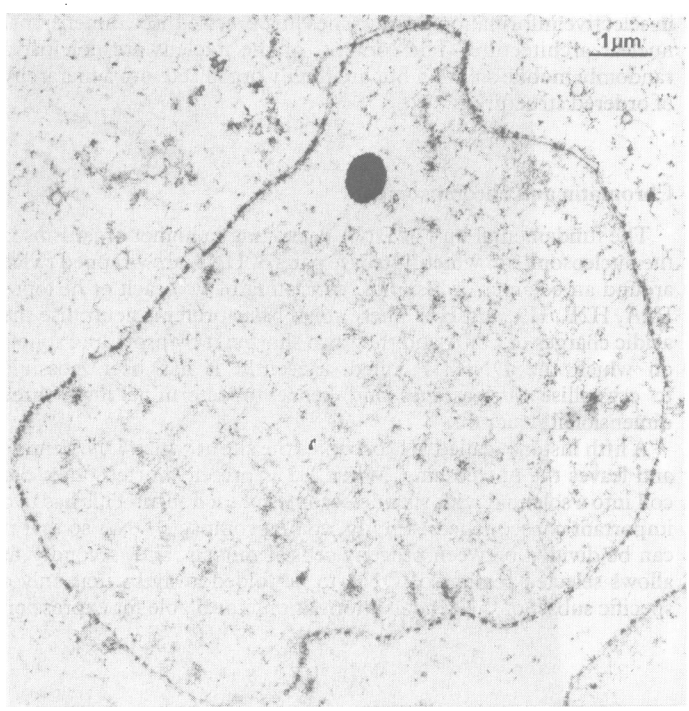

FIG 3—Electron micrograph of a section through a nucleus reconstituted from demembranated frog sperm chromatin by incubation in a cell free system which also assembles nuclei from naked DNA. (See Blow and Laskey (1986) for details. Photograph by courtesy of A D Mills.)

protein particles out of the nucleus, including ribosomes and messenger RNA. This process is also highly selective, resulting in the export of mature transcripts but the retention in the nucleus of immature transcripts. The basis of this selectivity is unknown, but in at least one case there is evidence of a carrier mediated transport system.

Rebuilding the cell nucleus

Recently there have been substantial advances in our ability to reconstruct "nuclei" from condensed chromatin or even purified DNA in cell free systems. Figure 3 shows a large fully enveloped nucleus which has been formed from demembranated frog sperm chromatin in a cell free system derived from frog eggs. Similar structures have now been formed from purified DNA, including DNA from bacterial viruses and plasmids. Not only is such DNA assembled into chromatin; it also becomes surrounded by nuclear membranes containing functional nuclear pores. Thus these pseudonuclei fill up with nuclear proteins and even replicate their DNA in vitro.

The opportunity to reconstruct functioning nuclei and functioning chromosomes opens up new approaches to understanding the complex structure and function of the cell nucleus.

Further reading

General

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. *Molecular biology of the cell*. Chapter 8. New York and London: Garland Inc, 1983.
Lewin B. *Genes III*. New York: Wiley, 1987.

Chromatin and chromosomes

Blackburn EM. Artificial chromosomes in yeast. *Trends in Genetics* 1985;1:8-12.
Earnshaw WC, Laemmli UK. Architecture of metaphase chromosomes and chromosome scaffolds. *J Cell Biol* 1983;96:84-93.
Klug A, Rhodes D, Smith J, Finch JT, Thomas JO. A low resolution structure for the histone core of the nucleosome. *Nature* 1980;287:509-16.

Gene expression

Dynan WS, Tjian R. Control of eukaryotic messenger RNA synthesis by sequence-specific DNA binding proteins. *Nature* 1985;316:774-8.
Serfling E, Jasin M, Schaffner W. Enhancers and eukaryotic gene transcription. *Trends in Genetics* 1985;1:224-30.
Harrison SC. Fingers and DNA half turns. *Nature* 1986;322:597-8.

Role of the nuclear envelope

Dingwall C. The accumulation of proteins in the nucleus. *Trends in Biochemical Sciences* 1985;10:64-6.

Rebuilding the cell nucleus

Blow JJ, Laskey RA. Initiation of DNA replication in nuclei and purified DNA by a cell-free extract of Xenopus eggs. *Cell* 1986;47:577-87.
Laskey RA. Prospects for reassembling the cell nucleus. *J Cell Sci [Suppl]* 1986;4:1-9.

Epidemiology

Report from the PHLS Communicable Disease Surveillance Centre

During the summer the epidemic of meningococcal meningitis receded, with notifications falling to their 1986 level, though a few infections were imported from Saudi Arabia; laboratory reports of respiratory infections due to parainfluenza type 3 rose, while those due to respiratory syncytial virus and *Mycoplasma pneumoniae* declined; there was an outbreak of campylobacter infection due to pasteurised milk, and the number of laboratory reports of both campylobacter and salmonellosis was higher than ever before for the same period of the year; and the cumulative total of cases of the acquired immune deficiency syndrome (AIDS) reported in the UK reached over 1000 by the end of August.

Meningococcal meningitis

Over 400 cases of meningococcal meningitis were notified in the first quarter of 1987 in England and Wales, almost twice the number seen in the same period in 1986 and about the same as the peak of the previous epidemic in 1974. Laboratory reports of meningococcal bacteraemia and meningitis showed the same trend, but, unlike the rise in 1985 and 1986, which was associated with an increase in reports of group B type 15 strains of *Neisseria meningitidis*, notably from the Gloucester area, the rise seen in 1987 was mainly due to group C strains. A seasonal fall was expected during the spring and summer, but notifications were thought likely to continue at about double the 1986 level. Surprisingly, however, the epidemic receded, notifications falling back to almost the same as 1986, closely resembling the pattern of the previous epidemic in 1974 (fig 1). If this pattern is followed in the next three years a further

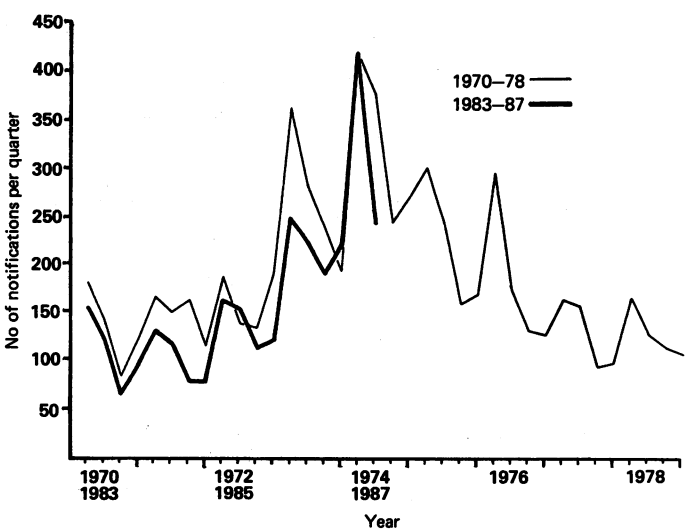

FIG 1—Meningococcal meningitis in England and Wales 1970-8 and 1983-7.

fall in notifications would be expected, reaching "normal" inter-epidemic levels in 1990. Even so, there will still probably be at least 400 notifications of meningococcal meningitis a year and as many cases of meningococcal systemic infections with or without meningitis which escape notification and about 80 registered deaths, over half of them in people aged under 20 years.

A less common event took place in August, when *N meningitidis*